

AWARD NUMBER: W81XWH-10-1-0089

TITLE: Adoptive Cellular Therapy Targeting Recurrent Pediatric Brain Cancers During Hematopoietic Recovery From High-Dose Chemotherapy

PRINCIPAL INVESTIGATOR: Duane A. Mitchell, M.D., Ph.D.

CONTRACTING ORGANIZATION: Duke University School of Medicine
Durham, NC 27705

REPORT DATE: April 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE 1 April 2011		2. REPORT TYPE Annual		3. DATES COVERED 1 Apr 2010 – 31 Mar 2011	
4. TITLE AND SUBTITLE Adoptive Cellular Therapy Targeting Recurrent Pediatric Brain Cancers During Hematopoietic Recovery From High-Dose Chemotherapy				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-10-1-0089	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Duane A. Mitchell, M.D., Ph.D. E-Mail: d.mitchell@duke.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Duke University School of Medicine Durham, NC 27705				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT A phase I/II clinical trial evaluating the feasibility, safety, and clinical efficacy of adoptive cellular therapy combined with dendritic cell (DC) vaccination targeting recurrent medulloblastoma and PNETs was opened for enrollment at Duke University Medical Center during the previous funding period (Year 1). DC generation and T cell expansion from archived post-induction chemotherapy specimens from five patients with medulloblastoma was attempted and mature RNA-pulsed DCs were successfully generated from 3 of 5 specimens and successful T cell expansion was achieved from these samples. Limitations in the yield of DCs and T cells from these specimens prompted a modification to the protocol to collect a leukapheresis sample prior to induction chemotherapy. A web-based clinical database for patient and laboratory data storage (Oracle Clinical) has been created by the Duke Comprehensive Cancer Center Bioinformatics team and is undergoing final beta testing and personnel training for release. Advertising efforts have increased awareness and recent referrals to the study protocol. Two subjects have been enrolled on the protocol, one removed due to final pathology other than medulloblastoma/PNET, and the other scheduled for surgical resection next week. Two additional subjects are currently undergoing screening for enrollment.					
15. SUBJECT TERMS Immunotherapy, medulloblastoma, PNET, dendritic cells, adoptive cellular therapy, clinical trial, phase I, phase II					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 25	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	3
Reportable Outcomes.....	7
Conclusion.....	7
References.....	7
Appendices.....	8

Section I- Introduction

Malignant brain tumors now represent the most frequent cause of cancer death in children. Despite aggressive and highly toxic multi-modality therapy including surgery, craniospinal radiation, and high-dose chemotherapy coupled with peripheral blood stem cell transplantation (HDC + PBSCT), almost half the children diagnosed with the most common malignant brain tumors, medulloblastoma and primitive neuroectodermal tumors (MB/PNETs), will still die from recurrent disease. Furthermore, survivors are often left with severe and lifelong treatment-associated cognitive and motor deficits. The development of more effective and tumor-specific therapies that will not add further toxicity to existing treatments is paramount in improving clinical outcomes for children affected by MB/PNETs. Immunotherapy targeting tumor-specific antigens expressed within brain tumors is a modality potentially capable of meeting this clear and urgent need. The use of total tumor RNA-loaded dendritic cells (DCs) was pioneered at our institution as a novel platform for inducing potent immunologic responses against the variety of uncharacterized and patient-specific antigens present within malignant tumor cells. The goal of this research is to establish the feasibility, safety, and estimate the clinical efficacy of adoptive cellular therapy targeting recurrent MB/PNETs using amplified total tumor RNA pulsed DCs to expand tumor-specific T cells *in vitro*. Adoptive T cell transfer coupled with intradermal DC vaccination will be performed in pediatric patients during the recovery from high-dose chemotherapy and peripheral blood stem cell rescue. A single arm, phase I/II trial will evaluate the safety and utility of this novel treatment approach.

Section II – Body and Key Research Accomplishments

Patient Enrollment: The Re-MATCH protocol received final approval for opening on 7/9/2010 and significant effort has been expended by the study PI and Co-PIs on raising awareness and recruiting eligible patients for this trial. These efforts have been detailed in previous quarterly reports, but in summary include the presentation of the protocol at several pediatric oncology conferences with both national and international audiences. These include: Spring 2010 Pediatric Brain Tumor Consortium (PBTC) meeting, Fall 2010 PBTC meeting, 2010 Southeast Pediatric Oncology meeting, 2011 American Society of Pediatric Neurosurgeons meeting, 2011 Cure Starts Now Pediatric Brain Tumor Conference @ Cincinnati Children's Hospital, and 2011 Society of Neuro-Oncology & Pediatric Brain Tumor Foundation Meeting. In addition, advertisement flyers have been disseminated to all members of the Society of Neuro-Oncology (>900 members) through a purchase list-serve mailing list. We have also advertised our trial on our own Duke Brain Tumor Immunotherapy Program website, listed the trial on clinicaltrials.gov, and enlisted the help of the Pediatric Brain Tumor Foundation of the United States in listing our trial on the front of their organizational website. These efforts have significantly increased awareness of the Re-MATCH protocol as we have

begun to receive inquiries and referrals about the protocol from throughout the United States as well as abroad (China, France and Sweden). In the last three months we have enrolled 6 patients on the protocol with our tumor collection screening consent bringing our total enrollment in the first year to 7 patients. Two patients were removed from the protocol due to final pathology other than recurrent medulloblastoma or PNET, and one patient rapidly declined and died from tumor progression within 5 weeks of surgical resection and prior to receiving the study drug. The other four patients are in various stages of post-operative care, finalizing staging and eligibility determination, and initiation of post-surgical adjuvant chemotherapy. The induction regimens for patients enrolled on this trial span an average of four months so we anticipate the first infusions of ex vivo expanded T cells and DC vaccines in late September or early October time frame.

Additionally, we have modified the protocol to increase the age limit of enrollment to 30 yrs of age and include patients who are not candidates for high-dose chemotherapy but will receive salvage chemotherapy followed by a non-myeloablative conditioning regimen prior to adoptive cellular therapy. This cohort of patients (Group B) will undergo phase I evaluation in parallel with patients receiving high-dose chemotherapy (Group A). The amendment to include Group B patients has received Duke Cancer Protocol Committee, Duke IRB, and US Army HRPO approval, and awaits final approval by the FDA for implementation. We will update our advertisements to include these patients once final approval has been received.

We are very pleased with the results of these recruitment efforts and believe continued discussion and presentations at national meetings will allow us to reach the accrual goals for this protocol in the subsequent years of this study.

Database Management: Through a series of bi-weekly meetings we have completed the construction, beta-testing, and personnel training of an electronic database for the Re-MATCH protocol using Oracle Clinical software management solution. This database was constructed in collaboration with the Duke Comprehensive Cancer Center Bioinformatics development team with oversight by the study biostatistician, PI, and Clinical Laboratory Manager. The Re-MATCH Oracle Clinical database went “live” on July 15th after completion of training of all personnel who will need access to the database for data entry.

Protocol Amendments & Regulatory Issues: A report summary detailing original protocol submissions and amendment approvals is attached as Appendix I.

Immune Monitoring and Correlative Studies: We have validated our capacity to analyze lymphocyte populations (CD4+, CD8+, and CD4+CD25+FOXP3+ regulatory cells) from children with medulloblastoma using 2mLs of peripheral blood.

We have synthesized and received custom gene array chips from Agilent Technologies and submitted initial test sample RNA to our microarray facility from *in vitro* cultured cell lines to validate the performance of the gene chips. Initial data analysis of the custom chip revealed a subset of probes (~40 of the 209 custom probes) that did not meet performance standards for hybridization. We are currently troubleshooting with technical support of Agilent Technologies to redesign these probes in order to adequate performance for all probes on the custom array. Genes in which suitable probes cannot be designed will be removed from our custom array analysis plan, but since these correlative studies will be conducted retrospectively on archived samples we will continue to develop the antigen gene chip with a goal of high-fidelity hybridization across all identified targets of interest.

Tumor RNA amplification: We have successfully extracted and amplified tumor RNA from 2/2 attempted tumor specimens and are currently working on the amplification of RNA from the remaining tumor specimens that we have acquired under the screening and tumor collection consent. Our experience thus far has confirmed that the protocol we developed for RNA extraction and amplification from adult GBM specimens is readily applicable to pediatric MB/PNETs and is encouraging for the capacity to prepare sufficient material for vaccine preparation from limited quantities of tumor tissue.

Dendritic cell generation: We have demonstrated feasibility of DC generation from archived leukapheresis specimens from children with MB and shown phenotype and immunologic function suitable to pass QA/QC release criteria for clinical use. This analysis did reveal that yields of DCs and lymphocytes after induction chemotherapy would not be sufficient for generating the target number of DCs and T cells for this protocol and prompted a protocol amendment allowing for collection of PBMCs prior to the initiation of induction chemotherapy. This amendment (AMD #1) was approved by the Duke IRB on 6/6/2010.

Personnel Changes: Our previous study coordinator at the end of May 2011 to fill a different role within our medical center. A new study coordinator has been added to the protocol to replace this effort. Additionally, our medical monitor, Dr. Rebecca Haley, has taken a position outside of Duke and will no longer be able to fulfill the role of medical monitor for this study. We are currently identifying a suitable replacement for her role (primary liaison with the DSMB, reviewer of all reportable AEs and SAEs, review of annual reports to FDA and IRB). As policy, all oncology protocols within Duke Medical Center have ongoing data and safety monitoring by the Duke Cancer Institute Clinical Monitoring Committee, including an initial review after enrollment of three patients and annual monitoring. We therefore feel that sufficient external monitoring to ensure safe and regulatory compliant conduction of this single-site trial is in place during the interim in which a suitable medical monitor is identified.

Reportable Outcomes: 7 patients have been consented on the study using the tumor collection and eligibility screening consent form for the Re-MATCH protocol. Two patients were removed from the study due to final pathology for recurrence other than MB/PNET and one patient was removed for tumor progression prior to receiving adoptive cellular therapy. Tumor tissue has been collected from 5 patients undergoing post-surgical staging and eligibility screening. Tumor RNA extraction and amplification has been successful from 2 out of 2 evaluated specimens with work-up of remaining tumor specimens ongoing.

Conclusions: Increased recruitment efforts have significantly enhance screening and enrollment on the Re-MATCH protocol. A pending amendment to include patients with relapsed MB and PNETs that are not candidates for high-dose chemotherapy is expected to further increase enrollment. Full-length tumor RNA amplification to clinical-scale (milligram quantities) from resected recurrent MB and PNETs is feasible for use in RNA-pulsed dendritic cell based immunotherapy protocols.

References: None

Appendices

- I-** Re-MATCH Regulatory Summary
- II-** Inclusion Enrollment Report
- III-** FDA Annual Report

REMATCH: "Recurrent Medulloblastoma and Primitive Neuroectodermal Tumor Adoptive T Cell Therapy during Recovery from Myeloablative Chemotherapy and Hematopoietic Stem Cell Transplantation" under BB-IND 14,058

Report Summary

1. Duke University Health System's Cancer Protocol Committee (CPC) approved REMATCH on 8/6/2009.
2. National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC) exemption from in-depth review and public RAC discussion received 8/28/2009.
3. Duke University Health System's Institutional Biosafety Committee (IBC) approved REMATCH on 9/23/2009.
4. Duke University Health System's Institutional Review Board (IRB) approved REMATCH on 12/2/2009.
5. US Army Department of Defense (DoD) Human Subject Research Review Board (HSRRB) approved REMATCH on 6/13/2010.
6. Primary Investigator Duane A. Mitchell, M.D., Ph.D. notification of officially opened for enrollment sent 6/14/2010.
7. FDA approved BB-IND 14,058 on 7/9/2010.
8. AMD # 1 - Reconciliation amendment to the REMATCH Protocol incorporating changes since original submission that included:
 - Moving the first mobilization leukapheresis prior to induction chemotherapy,
 - Clerical changes,
 - Clarification of study procedures and design,
 - Changes requested by the DoD HSRRB.This was sent to the FDA and DoD HSRRB on 5/19/2010.
This was approved by the Duke University Health System's IRB on 6/9/2010.
9. AMD # 2 - Amendment to advertise for this study through brochures and flyers for both patients and health care providers approved by the Duke University Health System's IRB on 8/4/2010.
10. The 2010 Annual Report on BB-IND 14,058 was submitted to the FDA, DoD HSRRB, and to RAC on 8/9/2010.
11. The 2010 Continuing Review (Annual Report) was approved 10/5/2010 by the Duke University Health System's IRB.
12. AMD # 3 - Amendment to include a screening consent for tumor acquisition was submitted and approved by the Duke University Health System's IRB on 1/14/2011.
13. AMD # 4 – Amendment for screening consents to include cytology specimens and to include storage of tumor specimens pending final eligibility screening within the research summary. This was approved by the Duke IRB on 4/29/2011.
14. AMD # 5 – Amendment for an additional cohort (Group B) that aren't eligible for HDC, but will receive non-myeloablative salvage chemotherapy. This was approved by the Duke CPC on

6/23/2011 and the Duke IRB on 6/27/2011. This was approved by DoD HSRRB on 7/5/2011.
This was submitted to the FDA on 6/14/2011.

15. The 2011 Annual Report on BB-IND 14,058 was submitted to the FDA, DoD HSRRB, RAC and IBC on 7/19/2011.

Inclusion Enrollment Report

Study Title: Re-MATCH

Total Enrollment: 7

Protocol Number: Duke IRB 18020

Grant Number: W81XWH-10-1-0089

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race				
Ethnic Category	Sex/Gender			
	Females	Males	Unknown or Not Reported	Total
Hispanic or Latino	0	0	0	0 **
Not Hispanic or Latino	2	5	0	7
Unknown (individuals not reporting ethnicity)	0	0	0	0
Ethnic Category: Total of All Subjects*	2	5		7 *
Racial Categories				
American Indian/Alaska Native	0	0	0	0
Asian	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0
Black or African American	0	0	0	0
White	2	5	0	7
More Than One Race	0	0	0	0
Unknown or Not Reported	0	0	0	0
Racial Categories: Total of All Subjects*	2	5	0	7 *
PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)				
Racial Categories	Females	Males	Unknown or Not Reported	Total
American Indian or Alaska Native	0	0	0	0
Asian	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0
Black or African American	0	0	0	0
White	2	5	0	0
More Than One Race	0	0	0	0
Unknown or Not Reported	0	0	0	0
Racial Categories: Total of Hispanics or	2	5	0	7 **

* These totals must agree.

** These totals must agree.



DUKE UNIVERSITY MEDICAL CENTER

Duane A. Mitchell, M.D., Ph.D.

Assistant Professor of Neurosurgery

Associate Director, Duke Brain Tumor Immunotherapy Program

The Preston Robert Tisch Brain Tumor Center at Duke

Department of Surgery

Division of Neurosurgery

July 19, 2011

Gary E. Archer, Ph.D.
Clinical Laboratory Director
(919) 684-6977
gary.archer@duke.edu

Denise Lally-Goss, M.S.N., A.N.P.
Nurse Practitioner
(919) 684-3862
lally001@mc.duke.edu

Beth A. Perry, R.N.
Regulatory Affairs Specialist
Research Nurse Associate
(919) 684-9291
beth.perry@duke.edu

Shenell Summers
Staff Assistant
(919) 668-5370
shenell.summers@duke.edu

Lori Ellis
Staff Assistant
(919) 684-9041
lori.ellis@duke.edu

Eugene Rosenthal, Ph.D.
Biotechnology Program Advisor
Office of Biotechnology Activities
NIH
6705 Rockledge Drive, Suite 750
Bethesda, MD 20892
301-496-9838

Dear Dr. Rosenthal:

We are submitting the annual report on BB-IND-14,058 to all agencies.

I authorize and consent to the NIH Office of Biotechnology Activities using the protocol and other information submitted to it by Dr. Archer for our human gene transfer trial entitled, Re-MATCH: Recurrent Medulloblastoma and Primitive Neuroectodermal Tumor Adoptive T Cell Therapy during Recovery from Myeloablative Chemotherapy and Hematopoietic Stem Cell Transplantation (IRB # Pro00018020), including release of all such information at a public meeting of the Recombinant DNA Advisory Committee. This consent to public disclosure specifically includes information that was labeled "confidential" in the submission.

Sincerely,

Duane A. Mitchell, M.D., Ph.D.
Assistant Professor of Surgery
Duke University Medical Center

JHS:bp



DUKE UNIVERSITY MEDICAL CENTER

Duane A. Mitchell, M.D., Ph.D.

Assistant Professor of Neurosurgery

Associate Director, Duke Brain Tumor Immunotherapy Program

The Preston Robert Tisch Brain Tumor Center at Duke

Department of Surgery

Division of Neurosurgery

July 19, 2011

Gary E. Archer, Ph.D.
Clinical Laboratory Director
(919) 684-6977
gary.archer@duke.edu

Denise Lally-Goss, M.S.N., A.N.P.
Nurse Practitioner
(919) 684-3862
lally001@mc.duke.edu

Beth A. Perry, R.N.
Regulatory Affairs Specialist
Research Nurse Associate
(919) 684-9291
beth.perry@duke.edu

Shenell Summers
Staff Assistant
(919) 668-5370
shenell.summers@duke.edu

Lori Ellis
Staff Assistant
(919) 684-9041
lori.ellis@duke.edu

Mark L. Davidson RHIA
Regulatory Project Manager
Regulatory Management Staff
Office of Cellular, Tissue, and Gene Therapies
Center for Biologics Evaluation and Research
HFM-99, Room 200N
1401 Rockville Pike
Rockville, MD 20852-1448

Re: BB-IND-14,058, 1571(7)
REMATCH: Recurrent Medulloblastoma and Primitive Neuroectodermal
Tumor Adoptive T Cell Therapy during Recovery from Myeloablative
Chemotherapy and Hematopoietic Stem Cell Transplantation

Dear Mr. Davidson,

We are enclosing three (3) hole-punched, bound copies of the annual report on IND #14,058. Please feel free to contact Dr. Gary Archer at 919-684-6977 or myself with any questions or concerns.

Sincerely,

Duane A. Mitchell, M.D., Ph.D.
Assistant Professor of Surgery
Duke University Medical Center

JHS:bp

2011 Annual Report

IND 14058

Total tumor mRNA-pulsed autologous Dendritic Cells (DCs) (TTRNA-DCs) and tumor-specific *ex vivo* expanded autologous lymphocyte transfer (TTRNA-xALT)

Serial 0007

July 19, 2011

Confidential

Table of Contents

1	STUDY 1 INFORMATION	3
1.1	STUDY.....	3
1.2	ENROLLMENT UPDATE.....	4
1.3	BRIEF DESCRIPTION OF STUDY RESULTS	5
2	SUMMARY INFORMATION.....	5
2.1	ADVERSE EVENTS: FREQUENT AND SERIOUS	5
2.2	SUMMARY OF IND SAFETY REPORTS.....	5
2.3	STUDY SUBJECT DEATHS	5
2.4	STUDY SUBJECT DROPOUTS RESULTING FROM ADVERSE DRUG EXPERIENCES	5
2.5	UNDERSTANDING OF THE DRUG'S ACTION	5
2.6	LIST OF PRECLINICAL STUDIES	6
2.7	SUMMARY OF MANUFACTURING OR MICROBIOLOGICAL CHANGES.....	6
3	GENERAL INVESTIGATIONAL PLAN.....	6
3.1	BRIEF DESCRIPTION OF THE OVERALL INVESTIGATIONAL PLAN.....	6
3.1.1	<i>Rationale.....</i>	<i>6</i>
3.1.2	<i>Indication(s) to be Studied.....</i>	<i>11</i>
3.1.3	<i>Planned Clinical Trials.....</i>	<i>11</i>
3.1.4	<i>Estimated Number of Subjects</i>	<i>12</i>
3.1.5	<i>Anticipated Risks</i>	<i>12</i>
4	INVESTIGATOR BROCHURE.....	12
5	PROTOCOL MODIFICATIONS	12
6	FOREIGN MARKETING DEVELOPMENTS	12
7	OUTSTANDING BUSINESS WITH RESPECT TO IND	12

1 STUDY 1 INFORMATION

1.1 Study

Title of Study: **Re-MATCH: Recurrent Medulloblastoma and Primitive Neuroectodermal Tumor Adoptive T Cell Therapy during Recovery from Myeloablative Chemotherapy and Hematopoietic Stem Cell Transplantation (IRB # Pro00018020)**

Study Design: Phase 1/II

Purpose: The primary objective is to evaluate the safety of TTRNA-DCs and TTRNA-xALT (DC + xALT therapy) during recovery from HDC + PBSCT in pediatric patients with recurrent medulloblastoma and primitive neuroectodermal tumors (reMB/PNETs). Secondary objectives are to determine if DC + xALT therapy extends progression-free survival compared to historical controls, to determine the objective radiographic response rate of HDC + PBSCT coupled with DC + xALT therapy in patients with residual disease, to determine if the magnitude and persistence of anti-tumor humoral or cellular immunity correlates with clinical outcomes, to evaluate changes in cytokine profile and Toll-Like Receptor (TLR) activation status in pediatric patients with reMB/PNETs after HDC and during DC + xALT therapy, to characterize the immunologic phenotype of lymphocyte subsets (naïve, effector, memory, regulatory) and NK cells in patients with reMB/PNETs at diagnosis and throughout standard therapy and experimental therapy, to identify potential tumor specific antigens as vaccine candidates through characterizing the frequency of expression of the top 40 tumor associated antigens (TAA₄₀) identified in common malignancies in recurrent MB/PNETs, and to determine the overall survival rate in pediatric patients with reMB/PNETs receiving DC + xALT therapy after HDC plus PBSCT.

Patient Population: Children (age 0-30 years) with localized recurrent MB, pineoblastoma, and cerebral PNETs who suffer disease recurrence following standard radiotherapy +/- chemotherapy and who are candidates for HDC plus autologous stem cell rescue (Group A) or who are candidates for non-myeloablative salvage chemotherapy (Group B).

Study Status: Recently opened to enrollment. Due to the rare subject population and low accrual, we recently amended the study to include the Group B

patients. Patients will be followed for adverse events, radiographic and clinical progression, and survival once enrolled.

1.2 Enrollment Update

Study is opened to enrollment. Seven patients have been enrolled to date.

Table 1.2-1 Subject Demographics

Total Enrollment Report: Number of Subjects Enrolled to Date (Cumulative) By Ethnicity and Race

Ethnic Category	Females	Males	Unknown or not reported	Total
Hispanic or Latino	0	0	0	0
Not Hispanic or Latino	2	5	0	7
Unknown (Individuals not reporting ethnicity)	0	0	0	0
Ethnic Category: Total All Subjects*	2	5	0	7

Racial Categories	Females	Males	Unknown or not reported	Total
American Indian/ Alaska Native	0	0	0	0
Asian	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0
Black or African American	0	0	0	0
White	2	5	0	7
More than one race	0	0	0	0
Unknown or not reported	0	0	0	0
Racial Categories: Total of All Subjects*	2	5	0	7

Table 1.2-3 Status of Enrolled Participants

Total Enrollment	7
Total Treated	0
Total Eligibility Failures	3
Total Compassionate Treatments	0
Total Patient-specific Treatments	0
Total Moved to a Different Study	0
Total Treated	0
Completed treatment as planned (received study drug)	0
Received study drug but did not complete treatment due to disease progression	0
Total Treated	0
Followed until death	0
Alive and still being followed	0
Cause of Death	

**PD=Progressive Disease*

1.3 Brief Description of Study Results

None of the enrolled patients have received study drug yet.

2 SUMMARY INFORMATION

2.1 Adverse Events: Frequent and Serious

None

2.2 Summary of IND Safety Reports

No IND Safety Reports have been submitted to this IND.

2.3 Study Subject Deaths

One subject, who was considered an eligibility failure, died of progressive disease, prior to receiving study drug.

2.4 Study Subject Dropouts Resulting from Adverse Drug Experiences

None

2.5 Understanding of the Drug's Action

Only one dose of DCs is used in these studies. An escalating total dose of TTRNA-xALT ($3 \times 10^6/\text{Kg}$, $3 \times 10^7/\text{Kg}$, and $3 \times 10^8/\text{Kg}$) with TTRNA-DCs (2×10^7) will be evaluated in separate cohorts of 3 to 6 patients each for the purpose of establishing a maximally tolerated dose (MTD) in this patient population. These approved studies are not designed to derive information on pharmacokinetics or bioavailability.

2.6 List of Preclinical Studies

No preclinical or animal studies were performed for these protocols during the previous year.

2.7 Summary of Manufacturing or Microbiological Changes

There have been no manufacturing or microbiological changes made during the past year.

3 GENERAL INVESTIGATIONAL PLAN

3.1 Brief Description of the Overall Investigational Plan

This prospective Phase I/II clinical trial will evaluate the safety of autologous TTRNA mRNA-loaded T-cell immunotherapy in conjunction with TTRNA mRNA-loaded dendritic cell vaccine in patients with recurrent supratentorial primitive neuroectodermal tumors. Following surgical resection, biopsy, or cytology examination with confirmatory pathologic diagnosis, patients will be enrolled into HDC (Group A) or NMA salvage chemotherapy (Group B) based on eligibility for HDC. Patients with localized relapse and have not failed HDC+PBSCT previously will be enrolled into Group A as HDC+PBSCT is considered standard-of-care in the relapsed disease setting. Patients with disseminated disease, have previously failed HDC+PBSCT, or are otherwise considered poor candidates for HDC based on overall health status but otherwise meet eligibility criteria will be enrolled into Group B and will received a salvage chemotherapy regimen followed by non-myeloablative cyclophosphamide/fludarabine lymphodepletive conditioning prior to DC + xALT therapy.

Two phase I studies will be conducted concurrently using 3+3 study designs. One study will be conducted among patients who have received HDC with stem cell transplant after induction therapy (Group A), and the other study will focus on patients who receive NMA conditioning regimen after receipt of salvage chemotherapy treatment (Group B). Within each of these studies, an escalating total dose of TTRNA-xALT ($3 \times 10^6/\text{Kg}$, $3 \times 10^7/\text{Kg}$, and $3 \times 10^8/\text{Kg}$) with TTRNA-DCs (2×10^7) will be evaluated in separate cohorts of 3 to 6 patients each for the purpose of establishing a maximally tolerated dose (MTD) in this patient population. If DLT attributable to DC + xALT therapy is experienced in 1 of 3 patients, an additional 3 patients will be enrolled at the same dose. If none of 3 patients or 1 of 6 patients experienced DLT, dose will be escalated in subsequently accrued patients. If ≥ 2 patients experience dose limiting toxicity (DLT) attributable to the DC + xALT, the accrual to that Group will be halted. The immediate lower dose level will be considered the MTD and used in the Phase II portion of the study. A DLT will be defined as a vaccine-related Grade IV or any non-neurologic toxicity \geq Grade III toxicity of any duration. A Grade III neurologic toxicity will only be declared a DLT if not reversible

within 48 hours. Patients will be monitored for 2 weeks after the 3rd vaccine before considering the patient to have not experienced a DLT. Patients receiving less than 3 vaccines without DLT will be replaced for safety assessments. Although not considered a DLT, any patients with \geq Grade II urticaria will not receive further vaccines, will be withdrawn from the study, and will be replaced if less than 3 vaccines have been administered without DLT. Within each Group, the Phase I evaluation will enroll at least 3 patients who are evaluable for the determination of MTD, and a maximum of 18 patients. In light of patient withdrawal before the occurrence of DLT or the completion of 2 weeks of monitoring after the 3rd vaccine, a maximum of 22 patients will be accrued per Group.

The Phase II portion of this study will assess the clinical impact of DC + xALT therapy within either Group A or Group B, but not both. Study designs are described for both groups. However, only one study will be conducted with that being the group that is safe and more feasible to accrue. For the Phase II portion of the trial up to 42 patients will be enrolled to treat 35 patients with reMB/PNET with DC + xALT therapy.

Group A:

Patients enrolled into Group A will receive Induction chemotherapy that will include cyclophosphamide, etoposide, and temozolomide. Leukapheresis for autologous peripheral blood stem cells (PBSCs) will occur prior to and during induction cycles of chemotherapy and will continue until adequate quantities of nucleated cells are obtained. The leukapheresis products will be aliquoted for future autologous PBSC rescue during Consolidation and used to generate the TTRNA-xALT and multiple doses of the TTRNA-DCs for future use during Post-transplant Immunotherapy. After completion of Induction, eligible patients will proceed to Consolidation with high dose chemotherapy consisting of carboplatin, etoposide, and thiotepa, followed by autologous PBSC rescue. The following day, Post-transplant Immunotherapy begins with infusion of the TTRNA-xALT and injection of the first TTRNA-DC vaccine. During the Phase I trial, three dose levels of adoptive tumor-specific T cell immunotherapy will be evaluated with a fixed dose of TTRNA-DCs. During the Phase II trial, patients will receive TTRNA-xALT at the MTD with the fixed dose of TTRNA-DC. Patients will continue to receive TTRNA-DC vaccination monthly as an outpatient for up to one year if vaccine is available, toxicity is acceptable and no progression of tumor is noted.

Enrolled patients (3 to 18 patients for the Phase I trial and 35-42 patients for Phase II) with reMB/PNETs will receive 4 cycles of induction therapy consisting of two four week cycles of intravenous cyclophosphamide ($2 \text{ gm/m}^2/\text{day}$ for 2 days as Cycles 1 and 2) followed by two four week cycles of oral etoposide $30 \text{ mg/m}^2/\text{day}$ for 14 days plus oral temozolomide (TMZ) ($150 \text{ mg/m}^2/\text{d}$ for 5 days as Cycles 3 and 4). All patients will undergo mobilization with G-CSF ($5 \text{ } \mu\text{g/kg/d}$) until CD34+ PBSC count is $\geq 10 \text{ cells/}\mu\text{L}$. A leukapheresis will be obtained prior to the first induction cycle as well as after each induction cycle until enough peripheral blood stem cells (PBSCs) are harvested for autologous stem cell rescue and as a backup for rescue to a targeted dose of $2 \times 10^6 \text{ CD34+ PBSCs/kg}$ following high dose chemotherapy (HDC), and peripheral blood mononuclear cells (PBMCs) for TTRNA-xALT and TTRNA-DC generation. Patients who have radiographic or histologic evidence

of progression or who's DCs or xALT fail to meet release criteria will be replaced. For the purpose of this study, progression will be defined as a new lesion confirmed by biopsy or resection, positive cerebrospinal fluid (CSF) cytology, or a new metastatic lesion confirmed by biopsy or resection. Following recovery from the 4th cycle of induction chemotherapy ($\text{ANC} \geq 1000 \text{ cells}/\mu\text{L}$), patients will undergo disease restaging and organ function evaluation. Patients will be admitted to the Pediatric Bone Marrow Transplant Unit (BMTU) and receive Carboplatin (either $500 \text{ mg}/\text{m}^2$ or a dose calculated based on Calvert's formula and a AUC (Area under the concentration time curve) $7 \text{ mg}/\text{ml}/\text{min}$, whichever is less) on days -8, -7, and -6, followed by thiotepa $300 \text{ mg}/\text{m}^2$ and etoposide $250 \text{ mg}/\text{m}^2$ daily on days -5, -4, and -3. Three days after HDC (Day 0), patients will receive PBSC rescue. DC + xALT therapy will begin 24 hours after PBSC. These patients will receive TTRNA-xALT and TTRNA-DCs following the transplant as vaccine #1. Patients will receive G-CSF daily after PBSC rescue until ANC recovers to $>2000 \text{ cells}/\mu\text{L}$.

For phase II, the primary efficacy endpoint will be 12-month progression-free survival (PFS-12) rate after treatment with DC + xALT therapy as a surrogate for overall survival. Gururangan et al. reported that PFS-12 for patients with recurrent disease after initiating treatment with HDC + PBSC or standard salvage therapy was 33% (95% confidence interval of 10%, 59%) and two-year PFS of 0%. The population of patients that will be treated in the current protocol in Group A is similar to the subset of patients treated on the Gururangan regimen. Due to the complexity and expense of adoptive cellular therapy treatment, if the true PFS-12 associated with DC + xALT therapy is 33%, there would be limited interest in further investigation of this treatment regimen without significant modification to improve efficacy. However, if the true PFS-12 were 55% or greater, there would be interest in studying this treatment regimen within the context of larger confirmatory Phase II or III clinical trials. Therefore, the study is designed to differentiate between a PFS-12 rate of 33% (null hypothesis) and 55% (alternative hypothesis). With 35 patients enrolled in the Phase II trial, this assessment has 90% power assuming a type I error rate of 0.10. There is also 97% power to differentiate between PFS-12 rates of 33% and 60%.

Group B:

Patients enrolled into Group B will receive NMA salvage chemotherapy that will include cycles of etoposide, and temozolomide followed by cyclophosphamide and fludarabine. Enrolled patients (3 to 18 patients for the Phase I trial and 35-42 patients for Phase II) with reMB/PNETs will receive salvage chemotherapy consisting of 1-4 cycles of oral etoposide $30 \text{ mg}/\text{m}^2/\text{day}$ for 14 days plus oral temozolomide (TMZ) $150 \text{ mg}/\text{m}^2/\text{d}$ for 5 days monthly until Immunotherapy preparation is complete. A leukapheresis will be obtained prior to and/or during salvage chemotherapy cycles until enough peripheral blood stem cells (PBSCs) are harvested as a backup for autologous stem cell rescue to a targeted dose of $2 \times 10^6 \text{ CD34+ PBSCs}/\text{kg}$ and peripheral blood mononuclear cells (PBMCs) for TTRNA-xALT and TTRNA-DC generation. Patients whose DCs or xALT fail to meet release criteria will be replaced. For the purpose of this study, progression will be defined as a new lesion confirmed by biopsy or resection, positive cerebrospinal fluid (CSF) cytology (when previously negative), or radiographic progression as defined by protocol criteria.

After completion of NMA salvage chemotherapy and starting 8 days before Immunotherapy, patients will receive 2 days of cyclophosphamide I.V. at 1g/m^2 , followed by 5 days of fludarabine I.V. at 25 mg/m^2 . On the day after the final dose of fludarabine, Immunotherapy begins with infusion of TTRNA-xALT and injection of the first TTRNA-DC vaccine. Patients will receive G-CSF daily after DC + xALT therapy until ANC recovers to $>500\text{ cells/}\mu\text{L}$. If ANC has not recovered to $500\text{ cells/}\mu\text{L}$ by Day 21 after Immunotherapy then PBSC rescue using cells collected for backup will be initiated. During the Phase I trial, three dose levels of adoptive tumor-specific T cell immunotherapy will be evaluated with a fixed dose of TTRNA-DCs. During the Phase II trial, patients will receive TTRNA-xALT at the MTD with the fixed dose of TTRNA-DC. Patients will continue to receive TTRNA-DC vaccination monthly as outpatients for up to one year if vaccine is available, toxicity is acceptable and no progression of tumor is noted.

NOTE: Based on prior chemotherapy history and/or hematologic performance, patients enrolled on Group B receiving salvage chemotherapy may need to have adjustment to the planned chemotherapy regimen at the discretion of the study pediatric neuro-oncologist, Dr. Sri Gururangan (Co-PI). As no salvage chemotherapy regimens in the recurrent setting of MB or PNETs have been found to be curative or achieve significantly prolonged disease-free survival, we do not anticipate any clinical impact of adjustments to the planned regimen in assessing PFS endpoint. All chemotherapy regimens will be recorded and any deviation from the prescribed regimen above noted.

Within Group B, a good historical benchmark for this patient population receiving a standardized salvage regimen is not readily available. However, previously published studies from our center and others have shown that patients unable to receive HDC and receive salvage regimens for recurrent disease have poorer prognosis than patients in Group A with localized disease relapse receiving HDC + PBSCT. Therefore, we will conservatively use the benchmark described above for Group A. With that assumption, the sample size justification described above is also applicable to Group B.

Immune Monitoring:

Peripheral blood will be drawn at -24 hours, +24 hours, +48 hours, +72 hours, and weekly post vaccine #1 for six weeks for T cell kinetics. DCs will be given intradermally and divided equally to both inguinal regions. Vaccines #2 and #3 will occur at 2 week intervals following the first dose. Blood will be drawn monthly with vaccine visits until tumor progression.

Patients will be followed until tumor progression and/or death due to any cause. As part of standard care for these patients, upon tumor progression, participants may undergo stereotactic biopsy or resection. As this is not a research procedure, consent will be obtained separately. If tissue is obtained, it will be used to confirm tumor progression histologically and to assess immunologic cell infiltration and antigen expression profile in recurrent lesions.

Risk/benefit assessment

Potential Benefits

Based on experience with immunotherapy and our previous clinical trials, DC-based immunotherapy may be of benefit to subjects with malignant gliomas. Of course, because individuals respond differently to therapy, no one can know in advance if it will be beneficial in an individual case. The potential benefits may include reduction and/or remission of the subject's brain cancer. Because this procedure is experimental, it cannot be guaranteed that subjects will receive any benefit as a result of participating in this research study. The information collected in this research may help scientists better understand the mechanisms involved in the immune system's ability to fight cancer. If such an understanding comes from this research, then it may benefit society by furthering the development of improved treatment methods for human malignant brain tumors in the future.

3.1.1 Rationale

- 3.1.2 Vaccination and adoptive T cell strategies targeting unfractionated tumor antigens in humans in other contexts have been safe and effective. DC vaccinations targeting tumor antigens in children with MB and PNETs and other brain tumors have been shown to be feasible and safe. The prognosis for patients with recurrent MB/PNETs (reMB/PNET) particularly for those who have failed prior definitive craniospinal irradiation is dismal. High Dose Chemotherapy (HDC) + PBSCT has been used as a standard of care in recent years in recurrent disease but is seldom curative. Myeloablative and non-myeloablative (NMA) chemotherapy induces a profound lymphopenia that would be predicted to prevent the induction of effective immune response to anti-tumor vaccination. However, recent studies have shown, somewhat counterintuitively, that vaccination during recovery from profound but transient lymphopenia or the adoptive transfer of tumor-specific lymphocytes into lymphodepleted hosts leads to dramatic in vivo T cell expansion and potent immunologic and clinical responses to immunotherapy. The use of TTRNA-pulsed DCs to expand tumor-specific lymphocytes ex vivo may provide a source of lymphocytes that preferentially expand in this lymphopenic environment and serve as a source of responder cells to subsequent DC vaccination. We and others have successfully employed the use of DCs loaded with total tumor RNA as an innovative strategy to induce cellular immune responses against the repertoire of, as yet, largely uncharacterized antigens present in malignant brain tumors. Tumor cells from MB/PNETs are often limited and cannot be reliably isolated or propagated in sufficient quantity to serve directly as a source of antigen for use in human vaccination protocols. We have, however, been able to reproducibly amplify the RNA content from as few as 500 isolated tumor cells using RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) and in vitro transcription from amplified cDNA (complimentary Deoxyribonucleic Acid) templates to generate RNA libraries in sufficient quality and quantity for clinical scale immunotherapy trials. We have also demonstrated that subtractive hybridization of RNA from normal brain can be used to enrich for antigens expressed exclusively within malignant brain tumor cells and possibly increase the specificity and safety profile of total tumor RNA directed immunotherapy.

3.1.3 Indication(s) to be Studied

Systemic immunization with tumor-specific antigen-pulsed DCs is feasible, safe and effective therapy for patients with malignant brain tumors. The primary objective is to evaluate the safety of TTRNA-DCs and TTRNA-xALT (DC + xALT therapy) during recovery from HDC + PBSCT (Group A) or during recovery from NMA salvage chemotherapy in pediatric patients with reMB/PNETs (Group B) in pediatric patients with reMB/PNETs. A secondary objective is to determine the impact of DC + xALT therapy on progression-free survival and overall survival of pediatric patients with reMB/PNETs compared to historical controls.

3.1.4 Planned Clinical Trials

We are not planning any new studies at this time.

3.1.5 Estimated Number of Subjects

A planned forty-four patients will be treated with DC + xALT therapy on this trial (9 patients on the Phase I study and 35 patients for completion of the Phase II trial). None have been enrolled to date.

3.1.6 Anticipated Risks

There are minor risks associated with the experimental procedures. For leukapheresis there may be discomfort or a swollen bruise at the site of needle puncture or light-headedness fainting, vomiting, and rapid breathing. For dendritic cell injections minor side effects could include fever, chills, or headache. Major side effects could include allergic reactions with inflammation in the throat or lungs. Although the dendritic cell cultures are grown sterilely and are monitored for contamination, it is still possible for an undetected bacteria or fungus to be injected with the dendritic cells. This could cause fever, chills, drop in blood pressure, and/or life threatening infection. The risks associated with the injection of autologous lymphocytes for immunotherapy in humans are currently unknown. There may also be side effects and discomforts, which are not yet known. Alternatives to this study are further surgery, chemotherapy, radiation, in or outside of another research study protocol.

4 INVESTIGATOR BROCHURE

None

5 PROTOCOL MODIFICATIONS

- Amendment to include a screening consent for tumor acquisition was submitted and approved by the Duke University Health System's IRB on 1/14/2011.
- Amendment for screening consents to include cytology specimens and to include storage of tumor specimens in the research summary. This was approved by the Duke IRB on 4/29/2011.
- Amendment for an additional cohort of patients (Group B) that aren't eligible for HDC, but will receive non-myeloablative salvage chemotherapy. This was approved by the Duke IRB and CPC on 6/27/2011 and the US Department of Defense on 7/5/2011. This was submitted to the FDA on 6/13/2011.

6 FOREIGN MARKETING DEVELOPMENTS

None

7 OUTSTANDING BUSINESS WITH RESPECT TO IND

None